AD NUMBER

AD845896

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; JUL 1968. Other requests shall be referred to Commanding Officer, Fort Detrick, Attn: SMUFD-AE-T, Frederick, MD 21701.

AUTHORITY

BDRL ltr, 13 Sep 1971

TRANSLATION NO. 269

DATE: July 1968

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#269

Contributions to the quantitative analysis of ergot alkaloids.

by E. Thielmann, W. Lang and H. Kaiser.

Archiv der Pharmazie, 286: 379-387 (1953). (Partial translation).

Experimental part.

Discussed are the Extraction of the ergot alkaloids from a drug and purification of the drug extract and

500 mg powdered (sieve 5, DAB 6) ergot are briefly heated with an alcoholic tartaric acid solution and filtered through a wad of cotton. For complete extraction the powder present on the filter is twice more covered with 5 cc each of the heated solvent. The filtrate, turbid due to protein and other accompanying substances and stained reddish-brown owing to dyes, is freed of undesired substances by precipitation with zink acetate. A 10% aqueous zink acetate solution was used, with which the ergot extract was filled to 25 ml.

Extract solution: 20 ml 20% tartaric acid solution
50 ml methanol
30 ml distilled water.

Zink acetate solution: 10 g $(CH_3COO)_2$ Zn
90 ml distilled water.

Quantitative determination of total alkaloids:

The clear and nearly untinted alkaloid solution, obtained in the manner previously described, is used directly for the determination. 1 vol. part of the drug extract is mixed with 2 vol. parts of p-dimethylaminobenzaldehyde reagent and the resulting blue color is measured in the photometer after 15 minutes. In the choice of spectral filters in the phase photometer, filter S 61 with a center of gravity of 6,190 A proved to be superior to other filters, since it showed maximal extinction in connection with the tested dye solution.

The concentration of the colorimetrically measured alkaloid solution is established according to a calibration curve produced with pure alkaloid solutions of known concentration. Since the concentration of the measured dye solution, according to Lambert-Beer's law, is directly proportional to extinction in the case of equal thickness, the calibration curve is linear.

p-dimethylaminobenzaldehyde reagent:

120 g H_2SO_4 (spec. grav. 1.836)
35 g distilled water
0.2 g p-dimethyaminobenzaldehyde
0.1 g $FeCl_3 \cdot 6 H_2O$.

the quantitative separation of ergot alkaloids into ergotamine and ergobasine groups by means of paper chromatography.

1 ml of purified drug extract, assumed to contain about 0.02-0.04% total alkaloids, is applied to the starting line of a piece of filter paper (20 X 15 cm) Schleicher & Schuell No. 2043 b. This is done after several intervals with a calibrated pipette of 0.1 ml capacity, the solution being allowed to dry each time. Clamped together in the form of a cylinder, the paper is placed in the glass dish within the chromatographic container. The bottom of the dish must first be covered with water.(Flat-bottomed Mason jars of appropriate height may be used as chromatographic containers, equipped with a suitable glass dish (Petri dish) for the reception of the filter paper and the organic solvent. It is recommended to bore a hole into the jar's lid, so that a stationary, closable glass tube, seated in a stopper, may be introduced to the bottom of the dish.) After the interior of the container has been saturated with water vapor — it is recommended, for this purpose, to let the jar stand overnight — the glass tube may be opened and the solvent poured into the glass dish.

After numerous tests, a mixture of o-formic acid ethylester - spir. dil. - isobutyl alcohol in the ratio 5:9:2 was chosen as organic solvent. Simple formic acid ethylester may be used instead of o-formic acid ethylester; however, the R_f value of the ergotamine ergotoxin is reduced thereby, so that the separation is not as distinct in connection with larger amounts of alkaloid.

As soon as the solvent's front approaches the upper edge of the paper, it is removed from the apparatus and dried. The temperature should not exceed 50°C.

After drying, the separated water-soluble and water-insoluble alkaloids may be labelled on the basis of their fluorescent properties under UV light. Ergobasine fluoresces a shining blue, while the ergotamine and ergotoxin groups show bluish-white fluorescence. The marked zones are cut from the paper in strips and hung lengthwise into a vessel for elution. A trough with an attached plate was used for this purpose, permitting the simultaneous elution of at least 6 strips. Experience showed that a uniform elution was favored by an angle of 70° between the plate and the external wall of the trough (Fig. 3). Care was taken also to see that the vessel was constantly filled, so that the solvent may breach the space between the hanging strip, the surface of the liquid and the vessel's wall by capillary action. In addition, the portion of the paper touching the plate was covered with a glass slide,

which must be slightly narrower than the paper. If it is wider, the elution liquid will travel by capillary action next to the filter paper, but not through it. The alcoholic tartaric acid solution described above was used as elution fluid.

By following this test procedure, the alkaloids were eluted within 15-30 minutes, the dripping alkaloidal solution being collected in small calibrated cylinders. The amount of elution fluid depends on the amount of expected alkaloid; about 2 ml must be allowed for 50 γ, if complete elution is to be assured. An overly strong dilution was avoided with a view to the possibilities offered by colorimetric measurement.

The tartaric acid solutions of water-soluble and water-insoluble ergot alkaloids thus obtained were subjected to colorimetric analysis after the accomplishment of the color reaction as above.